High-resolution molecular analysis of the 1982-3 FMD epidemic in Denmark

Christensen, Laurids Siig; Normann, Preben; de Stricker, Karin; Rosenorn, S.

Published in:
Report of the Open Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease

Publication date:
2004

Document Version
Publisher's PDF, also known as Version of record

High-resolution molecular analysis of the 1982-3 FMD epidemic in Denmark

Laurids Siig Christensen[1], Preben Normann[1], Karin de Stricker[1] and Stig Rosenorn[2]*
[1] Danish Institute for Food and Veterinary Research, Lindholm, DK-4771 Kalvehave, Denmark
[2] Danish Meteorological Institute, Lyngbyvej 100, DK-2100 Copenhagen K, Denmark

Abstract
The present study was conducted as a contingency exercise in molecular epidemiology, in particular exploiting the quasi-species structure of FMDV strains, with the additional purpose of gaining knowledge of epidemiological scenarios after introduction to a disease-free, non-vaccinated area. Seventeen isolates collected during the epidemic involving 23 outbreaks - 22 outbreaks on the island of Funen and one outbreak on the island of Zealand - were analysed by full-length sequencing of VP1 and aligned with available VP1 sequences using ClustalX.
The Denmark 1982-3 isolates cluster together with O1 strains but differs significantly from vaccine strain sequences available. The isolates can be clustered in three groups (strains) that correspond perfectly with the patterns of putative contact transmissions as revealed during the epidemic. The distribution of sequence markers is indicative of common recombination events in the history of the 3 strains. One outbreak on the island of Zealand was due to a virus strain identical to a strain emerging in 4 outbreaks on a location of Funen and meteorological data revealed conditions allowing an airborne transmission over a distance of 70 km to have taken place.
The data complies with a model where emerging epidemics most often is due to the appearance of a multiplicity of strains. Three strains were introduced to Funen, most likely by airborne transmission, from the former German Democratic Republic (GDR), and one case was introduced to the island of Zealand by airborne transmission from Funen. The indication of common recombination events in the history of the 3 strains suggest a source in an area with endemic FMD and this complies with a hypothesis that a preceding epidemic in the former GDR was due to an introduction from Ukraine.

Introduction
After absence of FMD in Denmark since 1970, vaccination against FMD was prohibited from January 1977. However, during March 14 to May 4, 1982, FMD was diagnosed in 20 cattle herds and 2 pig herds in Denmark (Westergaard, 1982). Twentyone farms were located on the island of Funen and one was located at the south-west coast of the island of Zealand. Still adopting the policies of non-vaccination and depopulation of FMDV-infected premises, a single outbreak of FMD was diagnosed in a cattle herd on the island of Funen in January 1983 (Anonymous, 1983). Thus, this epidemic in Denmark resembles the one in the UK by appearing in an FMDV-free, highly susceptible population of cloven-hoofed animals but differs significantly in terms of dissemination of the infection after introduction. The present study was conducted as a contingency exercise in molecular epidemiology with the additional purpose of gaining knowledge of epidemiological scenarios after introduction to a disease-free, non-vaccinated area. The study, in particular was an attempt to exploit the quasi-species nature of FMDV strains for high-resolution molecular analysis of epidemiological dynamics.

Materials and Methods
Virus samples and cultivation
A total of 21 of the 23 outbreaks were confirmed during the epidemic by virus isolation on primary cell cultures, or by inoculation in baby mice. Seventeen of these isolates collected from different farms were available and included in the present study. All isolates were propagated on monolayers of primary calf kidney cells grown to confluency according to standard procedures.

Sequencing and phylogenetic analyses
RNA was purified from cell culture supernatants and sequenced using a Type O VP1 full-length sequencing method to be published elsewhere. According to this method sequences are edited by reading in both directions of the PCR products using the PCR primers. Overlapping PCR products result in an additional 2-fold sequencing to be included as raw data in the sequence editing process. Sequences most identical to the VP1 coding regions of the Denmark 82-3 epidemic was identified using Blast (Altschul et al., 1997). Multiple alignments and phylograms of the sequences generated during the present study and sequences retrieved from the Genbank sequence database were done with ClustalX (EMBL, Heidelberg, Germany, May 1994) (Thompson et al., 1997) using the default parameters and 1000 bootstrap replications. The aligned sequences were presented with GeneDoc (Nicholas et al., 1997) and dendrograms were visualized with TREEVIEW (Page, 1996), version 25.

Meteorological data

Meteorological data ware retrieved from weather charts recorded 6 times daily by the Danish Meteorological Institute.

Results

The Denmark 1982-3 isolates were found to cluster together with the O1 field isolates collected from Germany and the vaccine strain O1/FRG/Kaufbeuren/66 used as vaccine in FRG at that time (Beck and Strohmeyer, 1987). The isolates could be referred to three distinct populations as indicated by ellipses in Fig. 1 by a number of nucleotide differences consistently found between isolates of the three populations. The three populations are defined as distinct strains designated O/DEN/Funen/A/82, O/DEN/Funen/B/82 and O/DEN/Funen/83 and are suggested to represent individual introductions to Denmark (the O/DEN section of the names is omitted from the results and discussion of the present report). Within each of the strains a number of nucleotide differences are observed but these differences on several occasions could be identified among plaque clones of the same isolate and are considered to represent strain inherent diversities.

In Fig. 1 is also shown the epidemiological links as revealed by interviews of farmers conducted by the Danish Veterinary Service during the epidemic (Westergaard, 1982; Anonymous, 1983).

The relationship of Skelskor/22/82 with strain Funen/B/82 identified in northern Funen as indicated in Fig. 2 led to a hypothesis of airborne transmission from the herds in northern Funen. The meteorological conditions were reviewed for April 1982 on days with wind directions allowing such a transmission as shown in Table 1.

Discussion

The number of isolates of the present study is sub-optimal to define quasi-species structures of strains as they are to be composed of isolates from different farms based on the assumption that these farms were infected by the same strain. Yet, the revealing of epidemiological links by the Veterinary Service during the epidemic (Westergaard, 1982, Anonymous, 1983) and the sequence information as it presents itself makes the approach feasible in this case.

The identification of three populations representing three strains introduced to Denmark complies perfectly with the epidemiological links revealed during the epidemic (Westergaard, 1982; Anonymous, 1983) as shown in Fig. 1. However, the possibility that concurrent introductions to more than one farm of virus referred to any of the three strains cannot be excluded

Strain Funen/83 was collected from an outbreak 9 months after the last outbreak on Funen in spring 1982. Based on a number of observations and a potential reservoir being sheep introduced to the barn in the winter of 1982-3 it was anticipated that this outbreak was due to an introduction in spring 1982 (Anonymous, 1983). The farm was located in an area where two outbreaks were diagnosed in April 82 in pig herds from which no virus was isolated. Whether these three outbreaks represent separate introductions or only one remains an open question.

The questions if any of the three strains could have evolved from each other during a spread in Denmark should be addressed. Mutations emerging in single virus particles and accumulating in a population would expand the diversity of the population unless a bottleneck appeared and provided there was no selective advantage of such mutations. A Dn/Ds ratio of 0.5 found for the nucleotide differences between the variants do neither indicate any restraints nor a selective advantage of the differences and given a bottleneck appeared, the manifestation of a mutation in a major sub-population of a strain therefore would be relatively rare. Given the unique markers of each strain found among all isolates of that strain successive incidences of bottlenecks resulting in the manifestation of minority variants as a majority population of the resulting strain and mutations would have to take place and this renders a linkage of any of the three strains by mutational evolution unlikely in such a short time span. The other question is if any of the three strains could have evolved from another strain by bottleneck transmission of a pre-existing minority variant. Given that the three strains were introduced from epidemiologically related sources (to be discussed below) any variant observed in any of the three strains could be present in trace amounts in both of the other two strains. However, based on the striking coincidence that no such hypothetical minority transmission is seen but in those cases where no transmission routes were indicated (Fig. 1) such an explanation remains hypothetical.

Although the three strains could hardly have evolved from each other, the isolates share the highly specific markes of an inherent diversity in two nucleotide positions. This strongly indicates a common source or epidemiologically related sources. Furthermore, the combination of an apparent strict linkage in isolates of some markers different for each of the three strains and the sharing of hyper-

variable markers by the three strains suggest that they share in their evolutionary history common recombination events which implies that the source(s) of the strains is located in an area with endemic FMD, i.e. an area where animals incidentally become co-infected or super-infected with different strains. Contacts between the national veterinary services at that time revealed that an epidemic had emerged at the north-west coastal area of GDR approximately 3 weeks before the outbreak in Denmark. This epidemic assumingly emerged after the importation of large amounts of meat from Ukraine to the city of Anklam, GDR (Dr. E. Stougaard, former Chief Veterinary Officer of Denmark, Personal Communication). A spread from northern GDR to Denmark of FMDV strains originating in Ukraine complies perfectly with the deductions of the present study.

Vaccines against FMD used at that time were often inadequately inactivated and cases of FMD in Central Europe at the time were concluded to be sero-type O1 vaccine-related (Beck and Strohmayer, 1987). However, the characteristics and in particular the distinct differences between the three strains emerging in Denmark do not support a hypothesis of a common vaccine ancestry.

Long distance airborne transmission has previously been shown to incidentally play a significant epidemiological role in the introductions of Aujeszky's disease virus to the island of Funen as well as to other border areas of Denmark (Christensen et al., 1990; Christensen et al., 1993) and it is straightforward to suggest that the FMD epidemic in 1982-3 was also due to airborne transmission. We have no data to positively support an explanation of airborne transmission of FMDV to Funen but the conclusion that three strains were introduced almost concurrently render a number of alternative explanations less likely. An airborne transmission from northern parts of DDR would imply transportations over a distance of 125 - 160 km mostly over the sea, which is not unprecedented (Gloster et. al., 1982; Donaldson et al., 1982). The suggestion that the outbreak at the south-west coast of Zealand was caused by airborne transmission from the northern part of Funen is based not only on strain identity but also on meteorological data allowing airborne transmission to have taken place on two days where the putative source was shedding virus.

The present report basically confirms the transmission routes as suggested by the investigations carried out by the Danish Veterinary Service during the epidemic in Denmark in 1982-3 (Westergaard, 1982; Anonymous, 1983). Thus, contact transmission by a practicing veterinarian during the initial phase attributed to a respiratory infection with FMDV and by truck drivers collecting dairy milk or pigs for slaughter could explain all cases of secondary spread of the infection. Strict measures were imposed on veterinarians and truck drivers in Denmark during the epidemic in the UK in 2001 to avoid similar contact transmissions. Had such measures been implemented after the first suspicion of FMD during the epidemic in Denmark in 1982-3 it is assessed, based on the epidemiological dynamics as now revealed, that no transmission from foci of primary introductions would have taken place.

Authors conclusions

- The 1982-3 FMD-epidemic in Denmark was due to three introductions and one case of airborne transmission between two islands in Denmark
- The source was from an area with endemic FMD most likely transmitted via East Germany

Authors Recommendation

- A European contingency in molecular epidemiology is elaborated allowing high-resolution analysis within 1-2 days

References

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D.J. 1997. "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res* 25:3389-3402.

Anonymous. 1983. Report on a single case of foot-and-mouth disease on the island of Funen, Denmark, January 1983. The Danish Veterinary Service and State Veterinary Institute for Virus Research, Denmark.

Beck, E. & Strohmaier, K. (1987) Subtyping of European foot-and-mouth disease virus strains by nucleotide sequence determination. *J Virol* 61: 1621 – 1629.

Christensen, L. S., Mousing, J., Mortensen, S. Soerensen, K. J., Strandbygaard, S. B., Henriksen, C. A. & Andersen, J. B. 1990. Evidence of long distance airborne transmission of Aujeszky's disease (pseudorabies) virus. *Vet Rec* 127 (19): 471 - 474.

Christensen, L. S.; Mortensen, S.; Boetner, A.; Strandbygaard, B. S.; Roensholt, L.; Henriksen, C. A. and Andersen, J. B. 1993. Further evidence of long distance airborne transmission of Aujeszky's disease (pseudorabies) virus. *Vet Rec* 132: 317 - 321.

Donaldson, A.I., Gloster, J., Harvey, L.D.J. & Deans, D.H. 1982. Use of prediction models to forecast and analyse airborne spread during the foot-and-mouth disease outbreaks in Brittany, Jersey and the Isle of Wight in 1981. *Vet Rec* 110: 53-57.

Gloster, J., Sellers, R.F. & Donaldson, A.I. 1982. Long distance transport of foot-and-mouth disease virus over the sea. *Vet Rec* 110: 47-52.

Nicholas, K.B., Nicholas, H.B.Jr. & Deerfield, D.W. 1997. GeneDoc: Analysis and Visualization of Genetic Variation, *EMBNEWS* 4:14

Page, R.D. 1996. Tree view: an application to display phylogenetic trees on personal computers. *Comp Appl Biosci* 12: 357-8

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin & F., Higgins, D.J. 1997. The ClustalX windows interface: flecible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl Acids Res* 24: 4875-82.

Westergaard, J.M. (Ed.). 1982.. Report on the eradication of foot-and-mouth disease on the islands of Funen and Zealand, Denmark 1982. The Danish Veterinary Service, Copenhagen, Denmark.

Table 1: Meteorological conditions reported every 3 hours at the airport of Funen on days with wind directions along the putative route of transmission

Date	Wind velocity	Temperature	Relative humidity	Remarks
April 12	5-10 m/s	4-8^{o}C	48-73%	Clouded, unsteady wind velocity and direction
April 15	4-6 m/s	12-13^{o}C	48-62%	Clouded, unsteady wind velocity and direction
April 16	5-9 m/s	5,7-12,6	53-81%	Clear sky, unsteady wind velocity and direction
April 17	3-9 m/s		28-92	Partly clouded to clear sky, Steady wind, variable humidity
April 19	1-2 m/s	2,7-8,8	65-84%	Clouded, no wind
April 21	1-4 m/s	4,2-6,4^{o}C	70-90%	Clouded, steady wind velocity and direction
April 24	1-4 m/s	3,1-4,8^{o}C	89-92%	Rain, no wind
April 25	1-7 m/s	3,5-11,9^{o}C	57-95%	Variable conditions
April 26	7-8 m/s	6,2-8,1^{o}C	75-87%	Windy
April 27	2-9 m/s	2,3-15,8^{o}C	50-91	Clear sky and variable humidity - otherwise periods of steady wind velocity and direction
April 28-30	5-13 m/s	2,3-10,8	41-91%	Windy

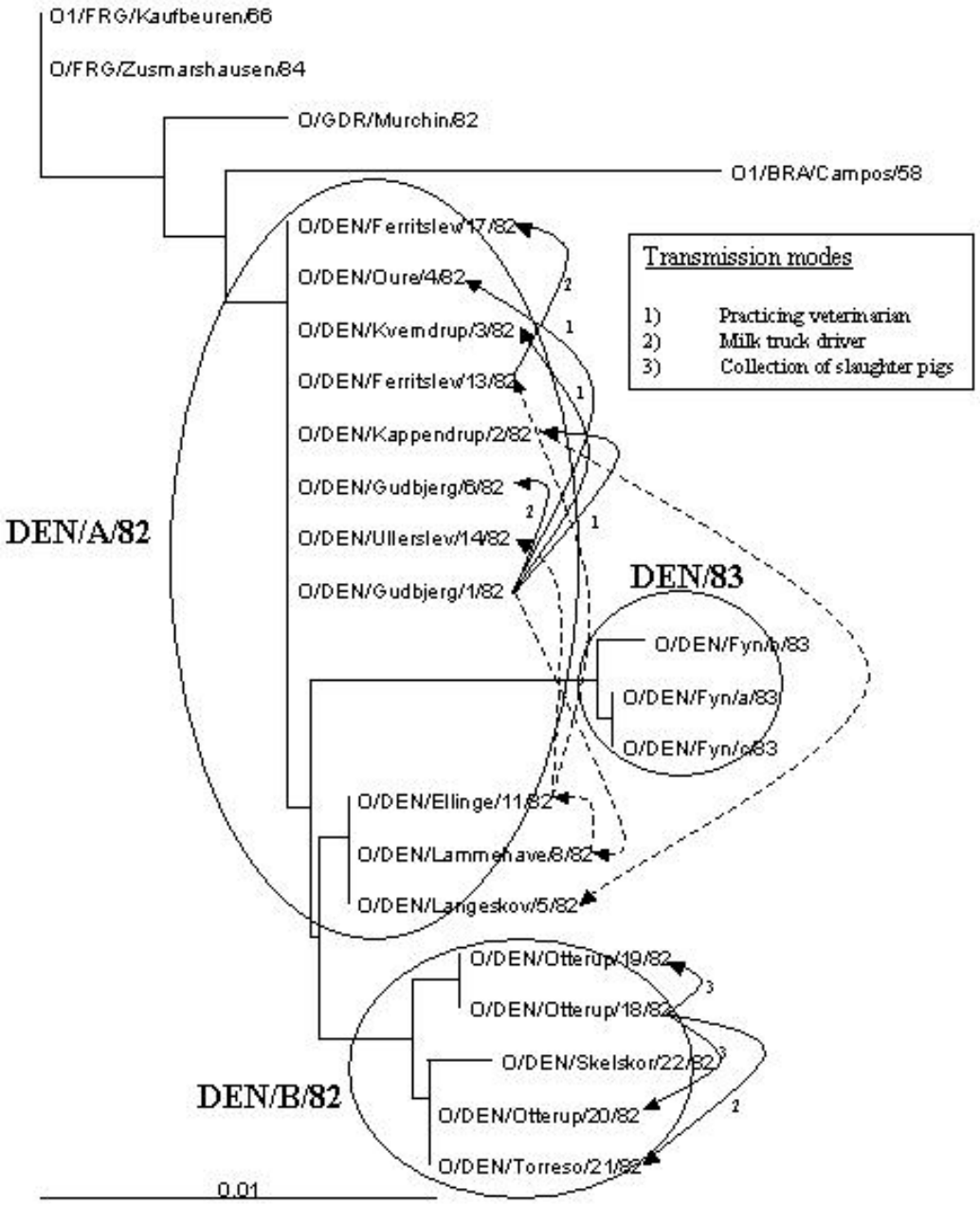

Figure 1. High-resolution dendrogram based on the alignment of VP1 sequences of all isolates from the outbreaks in Denmark in 1982-3 with VP1 sequences of other O1 FMDV strains. Putative strains are indicated by ellipses and putative transmission links as revealed by the Danish Veterinary Service during the epidemic (Westergaard, 1982; Anonymous, 1983) are also shown. Full lines indicate links of high validity and dashed lines indicate links of lower validity.

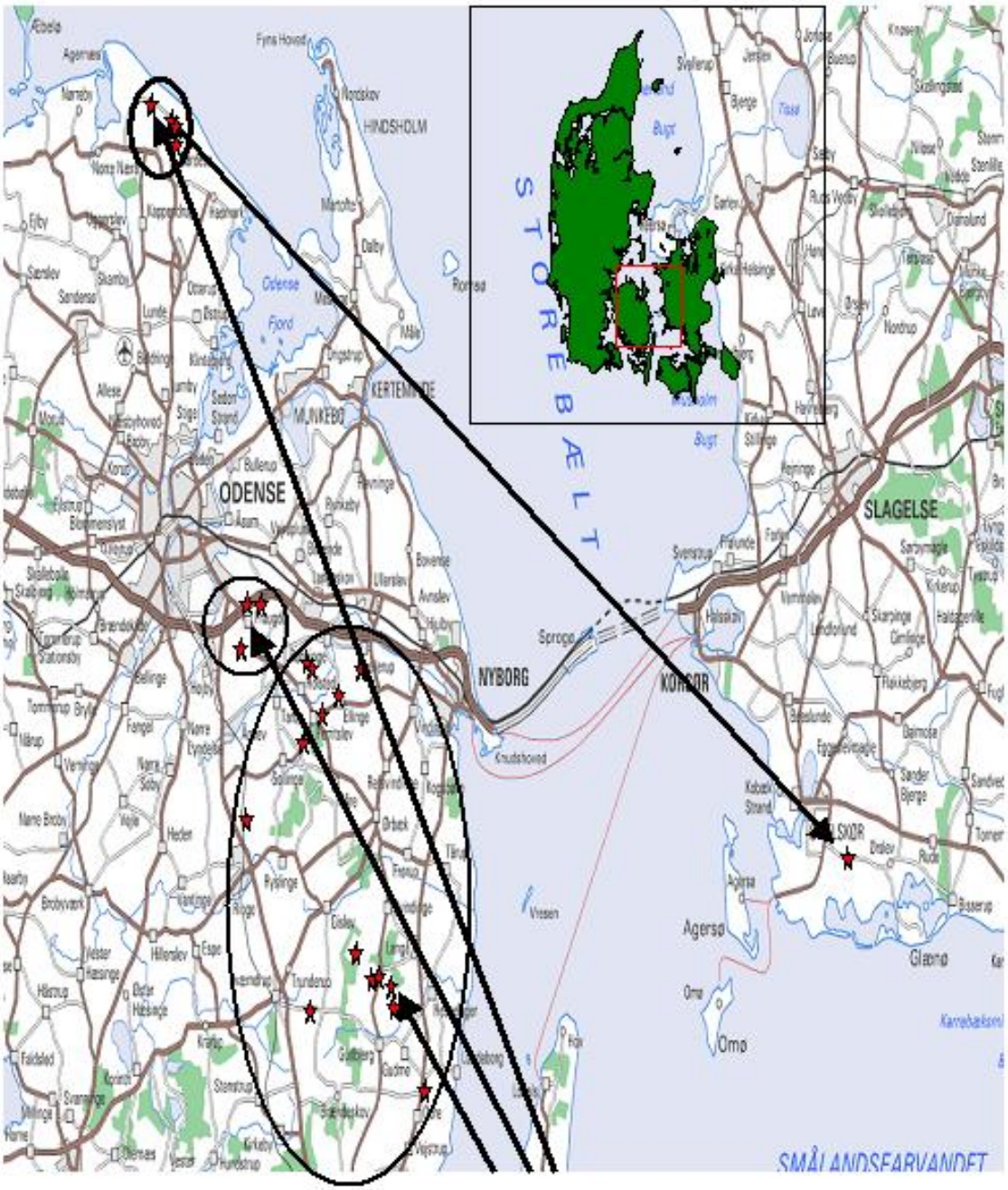

Figure 2. Localisation of the outbreaks of foot- and mouth disease in Denmark in 1982-3. Putative primary introductions and the case of airborne transmission from the island of Funen to the island of Zealand are indicated by arrows.